Biophotonic Sensing Cells Optimization for label-free biosensing

Optimización de Celdas Biofotónicas para detección óptica sin marcaje

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RESUMEN:

Los sectores de detección biológica demandan continuamente técnicas de análisis y diagnóstico más eficientes y precisas para identificar enfermedades y desarrollar nuevos medicamentos. Actualmente se considera que hay una gran necesidad de desarrollar herramientas de diagnóstico capaces de asegurar sensibilidad, rapidez, sencillez y asequibilidad para aplicaciones en sectores como la salud, la alimentación, el medioambiente o la seguridad.

En el ámbito clínico se necesitan profundos avances tecnológicos capaces de ofrecer análisis rápidos, exactos, fiables y asequibles en coste y que tengan como consecuencia la mejora clínica y económica a partir de un diagnóstico eficiente. En concreto, hay un interés creciente por la descentralización del diagnóstico clínico mediante plataformas de detección cercanas al usuario final, denominadas POCs (Point Of Care devices). La utilización de POCs (referidas al diagnóstico cercano al usuario final o fuera del laboratorio de análisis clínico), mediante detección in vitro (IVD), será extremadamente útil en centros de salud, clínicas o unidades hospitalarias, entornos laborales o incluso en el hogar. Por otra parte, el desarrollo de la genómica, proteómica y otras tecnologías conocidas como "omics" (sufijo en inglés para referirse, por ejemplo, a genomics, transcriptomics, proteomics, metabolomics, lipidomics) está incrementando la demanda de nuevas tecnologías mucho más avanzadas con una clara orientación hacia la medicina personalizada y la necesidad de hacer frente a cambios en los tratamientos en el caso de enfermedades complejas.

Desde hace poco tiempo se han definido las Celdas Biofónicas (BICELLs) como un metodología novedosa para la detección de agentes biológicos que ofrecen una serie de características que las hacen interesantes como son: Capacidad de multiplexación, alta sensibilidad, posibilidad de medir en gota, compatible con otras tecnologías. En este trabajo se hace un estudio y optimización sobre diferentes tipos de BICELLs y se valoran una serie de figuras de merito a tener en cuenta desde el punto de vista del lector óptico a emplear.

Palabras clave: Biosensores ópticos, Detección sin marcaje, Optimización, BICELLs.

ABSTRACT:

Label-free immunoassay sector is a ferment of activity, experiencing rapid growth as new technologies come forward and achieve acceptance. The challenges can be broken down into several key aspects as: sensibility and competitive limit of detection, ability of measuring molecules with different molecular mass, low reagent consumptions and the capability of measuring with simple drops for measuring different molecules simultaneously. The landscape is changing in a "bottom up" approach, as individual companies and research groups promoting key remarkable technologies. However, no many technologies based on Label-free technology are currently in the market, where mature labeled technologies such as lateral flow for its simplicity and ELISA for its sensitivity as ones of the most employed nowadays. Biophotonic Sensing Cells (BICELLs) defined as Bio-Sensitive cells that can be integrated in wells and interrogated vertically by enhanced optical techniques is currently an interesting approach for measuring biological agents as an label-free alternative for its capability of multiplexing many sensing sites in a single well, and therefore multiples diagnostics can be implemented simultaneously. Furthermore, good figures of sensitivity have been demonstrated for measuring different biological agents such as proteins, hormones and viruses, even in real samples. Several BICELLs type have been proposed and published. A good example it is that based on network of nanopillars made of low-cost resist, where bioreceptors can be immobilized easily. BICELLs technology is an interesting technology to face the unmet need for reliable diagnostic tools that ensure sensitive, rapid and simple analysis through a simple PoC reader.

Key words: BICELLs, Label-free biosensing, Optical detection.

1.- Introducction

Optical biosensing has focused high interest during past years. These sensors allow the detection of a huge diversity of bioanalytes by measuring variations of refractive index induced by molecular binding. In particular, detecting non-labeled analytes have particular interest, mainly due to the simplicity of the sensing protocol, compared with labeled sensors. The most developed tools are based on Surface Plasmon Resonance (SPR) principle broadly reported in the scientific literature as well as interferometry using for example Mach-Zehnder, ring resonator interferometers, among many others operating with a variety of waveguides for the sensing optical readout. The complexity of coupling the light from a fiber to these waveguides, and also for taking the bioanalyte to the sensing surface, by means of complex microfluidic circuits, it might be often considered to be a drawback, although promising results are continuously improving these types of biosensors.

In recent works we demonstrated a transducing methodology for label-free biosensing based on BICELLS and straightforward vertical optical light coupling systems by performing two different immunoassays: BSA/anti-BSA and Gestrinone/anti-Gestrinone. The obtained results were satisfactory, with reached limits of detection at a competitive level (2.3 ng/mL for antiBSA detection, and 64 pg/mL for antiGestrinone detection). Other advantages for this system are: the abovementioned light coupling system, the process of bio-functionalizing surfaces by simply putting a droplet of sample on the sensing surface, and the high multiplexing capability for measuring multiples analytes.

The principle of sensing for the biophotonic sensing cells is the following: the device consists of a periodic lattice of micro-nano pillars built on a silicon or glass substrate with, or without, an interferometric layer chosen among SiO2 for Si as substrate or Indium Tin Oxide (ITO) for glass as substrate.

In previously performed FDTD simulations we considered each pillar as a micro-nano metric sensor, but the optical readout is collecting the contributions of all of the pillars forming the BICELL. Thus, the light reflection or transmission of this lattice can be analyzed by spectrometry for monitoring both: the immobilization of bioreceptors onto the sensing surface and the biomolecular binding. The combination of micro-nano pillars network and the multilayer film stack offer a particular interference sensing curve, which dips and peaks shifts when the sensing surface (in this case, the pillars) are covered with a biofilm (which is equivalent to the variation of their refractive index).

The optimization of BICELLs requires a study of the effect of multiple input parameters on the model outputs, each one in range of values given by our experience or the limitations of the methods of fabrication of the sensitive cells. Parameters as the diameter and pitch of the pillars are studied, as long as the type of substrate and the refractive index of the pillars.

Past studies suggested that the variation on any of the design parameters of the periodic lattice has an influence in the expected shift of interference, and thus in the biosensing performance of the device .The goal of this work is define this design process, by identifying which are the most important parameters and state its influence in the final performance. To do this, a newfangled methodology based on an analytical simplified theoretical model is presented, simulated, studied, tested and compared with experimental and alternative theoretical results. The objective is to demonstrate that this model can be used for simulating complex biophotonic systems, and how this methodology can be used for optimizing these complex systems.

Thus, we have studied six different combinations of materials for the pillars and the underlying stratified structures in order to obtain more information about BICELLs behavior. Cases are shown in Fig. 1. These structures combine two different substrates, of silicon and glass and two different materials for the pillars (Silicon and SU-8). Diameter of the pillars and lattice parameter are varied in a range of values in order to see the influence of these change in the performance of the sensor.

Fig. 1. Micro-nano patterning structures: A) pillars over SiO2 substrate, the optical image represents a number of BICELLs made of SU8 nano-pillars over transparent substrate, B) pillars over Si substrate, the SEM image is a BICELL based on SU-8 nano-pillars, and C) pillars over Si as substrate with and interference SiO2 layer, the SEM image are a corner of a BICELL based on Si nano-pillars.

2.- BICELLs optimization

The second goal of this article is to show how this model can be applied for optimizing biophotonic-sensing structures. The typical optical response in these typologies of sensor is exposed in Fig. 6. The reflectivity spectrum has several maxima and minima produced by interference profile between the different layers. These interferences shift as the bioanalytes attach onto the sensing surface, as shown in the figure. The parameter used to evaluate the performance of the sensor is usually the total interference or resonance shift (λshift) strongly connected with the sensitivity of the biosensor. For tis work, we have considered that not only λshift is important, but also other aspects such as the signal Amplitude and the Full Width at Half Maximum or Minimum (FWHM) are important as drawn in the Fig.6.

Fig. 6. Shift of the optical resonance when covering the sensing surface of the pillars with a biofilm.

There are several methods for theoretically calculating the performance of the sensor; the magnitude most commonly used is the limit of detection (LOD). For optical biosensors LOD can be calculated in terms of refractive index, as the minimum variation of refractive index on the surface of the sensing area that the system is able to resolve. LOD values are calculated considering the relation between the spectral shift and the variation of refractive index, and also the uncertainty of the measurement of the spectral shift.

Thus, the usual strategy to optimize a biosensor is focusing on the maximization of this spectral shift. However, as some authors have pointed out [13], not only is important this magnitude, but also other aspects, such as the shape of the resonance, and the signal to noise ratio of the spectrum measured, among others. Therefore, the three figures of merit chosen for this work are defined with the Eq. (5), (6) and (7), which give information about the dip or peak signal width, signal amplitude (height) and wavelength displacement when adding a biological layer:

Sensing Quality Factor (SQ- Factor) = λshift / (Biolayer thickness) Eq.(1)

Amplitude Factor (A-Factor) = (Rmax - Rmin) x 100 Eq. (2)

Quality Factor (Q-Factor) = λresonance / FWHM Eq. (3)

For instance, in our previous work, for a biolayer of 16.5 nm (BSA-antiBSA) we reached a shift of 12.2 nm, the sensing quality factor has a value of 0.74 (nm/nmbiolayer). A higher value of SQ-Factor represents a better sensing performance, and it is goal of the optimization. The values of Q-factor and A-factor for previous experiments are 108 and 49.7, respectively.

Finally, the calculations have been performed for the six different configurations, or BICELLs type, presented in Fig. 1, and with two different calculations for each sensing cell: The reflectivity with no biolayer (reference spectrum), and the reflectivity with the sensing cell covered with an uniform biofilm of 20 nm and 1.4 in refractive index. With these six configurations several design parameters can be analyzed. First of all, it is the material of the pillars. Although the previous experiments with SU-8 presented good results, other materials are also interesting, in particular silicon, due to its special optical properties and also the possibility of reaching a highly compact pillars sensing cell.

Secondly, the material of the substrate also has its influence on the final results. Using glass instead of silicon wafer is a possibility, which moreover allows the possibility of measuring in transmission instead of reflection of light. Critical parameters in the fabrication of the cells are the diameter and the lattice parameter (or pitch) of the pillars. As a general rule, a higher compacting of pillars resulted on better values of LOD and sensitivity [9], but it makes also more complex the fabrication process. Finally the introduction of extra interferometric layers, for example of SiO2 does not complicate the fabrication process, but can represent a gain in terms of several of the figures of merit analyzed.

Summarizing, we have studied a total of six configurations; SU-8 pillars over a glass substrate, and SU-8 over silicon substrate, and SU-8 over a extra layer of 500 nm of SiO2 over silicon. These three configurations have been replicated but using Silicon pillars instead of SU-8. For each configuration, several combinations of pitch and diameter are studied.

3.- Resultados y discusión

The comparison between SU-8 and silicon pillars shows that with both materials good results for the three figures of merit can be reached. SQ-factors up to two are reached for silicon pillars over silicon, representing an important improvement compared with previous results (SQ-Factor of 0.74). However, with glass substrates both Q-Factor and especially A-Factor have low values due to the lower refractive index contrast. The resonances in these cases have low amplitude, and this makes recommendable the use of silicon substrates. An undesired increment in the Signal to Noise ratio of the spectral measurement can make the resonance undetectable. The same conclusion can be extracted also for SU-8 pillars over silicon, in which, though A and Q factors increase compared with glass substrates, still remain too low for a proper detection. In contrast, the silicon pillars over silicon substrate configuration allows reaching good values

for the three figures, and can be considered a good alternative to SU-8.

The introduction of a 500 nm SiO2 produces an important gain in the amplitude of the peaks of interference for SU-8 pillars, with values of A-Factor of 35, but reducing also values of SQ-Factor. If the thickness of this layer increases, this trend could be confirmed, with even lower values of SQ-Factor and higher of A-factor, as found with previous results (0.75 and 49 for an extra layer of 1 μm). There should be a thickness in which an optimal solution between SQ-Factor and A-Factor is found, and thus this is an interesting optimization for the future. However, for silicon pillars, there is not much improvement coming from the presence of an extra layer, and the performance even worsen, since the gain in terms of A-Factor is low compared with a higher loss of SQ-Factor.

A general conclusion for all the configurations is that having diameters and pitch among pillars as small as possible, results in better figures for SQ-Factor, Q factor and A-Factor. This is coherent with previous estimations, and must be the guideline for future BICELLs design. However, the fabrication process generally gives the limit of compacting for improving the biosensitive cell. Any advance in this field will lead to a better sensing performance. In contrast, structures with diameters and pitch up from 1.5 μm present poor values for the six configurations.

The calculated figure maps also allow taking some other conclusions about the BICELLs. It is not only important to obtain an optimized sensitive cell, but also achieving a robust design, in which slight changes in the dimensions of the pillars do not provoke important changes in the performance of the sensor. This has particular importance considering fabrication process of sub-micrometric pillars, in which the fabrication tolerances may be still significant with deviations from the nominal values that can be easily in the order of 10-20 per cent. For example, in Fig. 12 the SQ-Factor reaches its maximum for a wide range of values, whereas in Fig. 8, though this maximum has a similar value, a small variation in pitch will represent a decrease of SQ-Factor down to the half

4.- Conclusions

Two main general conclusions can be obtained for this work. Firstly that we have presented a simplified 1D model for calculating complex 3D micro-nano patterning materials, characterized vertically by spectrometry, and designed for biological sensing. This 1D analytical method has presented good correlation with previous experimental results, and allows obtaining results nearly as accurate as other complex simulation methodologies, such as 3D FDTD algorithm, but with a great gain in terms of time. Secondly, how this method can be applied for optimizing biophotonic sensing architectures. For tis purpose, six different configurations of BICELLs are studied, in each varying diameter, pitch and height of the pillars, calculating for each of them three different figures of merit.

Several conclusions can be extracted from the optimization process. First of all, the sub-micro nano scale increases the performance of the sensor, in contrast with pillars up from the micrometric scale. Moreover, reducing the pitch improves the three figures of merit. Secondly, using glass substrates is not recommended for both silicon and SU-8 pillars, since the amplitude of the resonances obtained is too low. In the case of SU-8 pillars, the presence of an extra interferometric layer allows increasing the amplitude of the dips, but with a cost in terms of SQ-Factor values; the same cannot be said for silicon pillars, where the results are better without the presence of this extra layer.

Finally, the short time of computation of the 1D model proposed has allowed the calculation of multiple combinations of parameters. The design of an optimized biosensor is a complex process, in which other aspects must be considered; in particular the fabrication (feasible BICELLs) and the biofunctionalization (taking bioanalytes to the sensing surfaces). This tool is useful for making a complete analysis in

the design process, in order to reach an optimized BICELL that also accomplish with requirements coming from fabrication and functionalization steps.

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